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# Molecular Assessment and Taxonomic Status of the Rapid Racerunner (*Eremias velox* complex) with Particular Attention to the Populations in Northwestern China

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**Abstract** The rapid racerunner, *Eremias velox*, is a widely distributed lizard from the northern Caucasus across entire Central Asia eastward to China. It is increasingly common to accept *E. velox* as a species complex in its entire range. To date, published morphological and molecular systematic hypotheses of this complex are only partially congruent, and its taxonomic status and evolutionary history are still far from clear. The mitochondrial cytochrome *b* gene and 12S rRNA sequences were used to evaluate the taxonomy of this complex, with particular attention to the phylogenetic placement of populations in northwestern China. Examination of the phylogenetic analyses recovers seven distinct, biogeographically discrete, and well-supported clades, revealing genetically identifiable populations corresponding to some previously morphology-defined subspecies. Chinese *E. v. roborowskii* appears to have split from other Central Asian rapid racerunner lizards well before differentiation occurred among the latter taxa. Specifically, we corroborate that there are two subspecies occurring in China, i.e., *E. v. velox* and *E. v. roborowskii*. We recommend a novel subspecific status for the phenotypically and genetically distinct populations in southern Aral Sea region of Uzbekistan previously assigned to *E. v. velox*. Finally, each of the three independently evolving lineages from Iranian Plateau should be recognized as three species new to science under the general lineage concept.

**Keywords** mtDNA, subspecies, *Eremias velox* complex, taxonomy, phylogeography

## 1. Introduction

Racerunner lizards of the genus *Eremias* (family Lacertidae) are the dominant reptiles in the deserts and steppes of Central Asia. The taxonomy of this genus has a long complex history, and the delimitation of species/subspecies of *Eremias* is known for being notoriously difficult. This is, in part, caused by the great geographic variation in most morphological characters in many of the species (e.g., Szczerbak, 1974, 1975; Ananjeva *et al.*, 1998; Chirikova, 2004). Currently, over 36 species have been assigned to the genus, and much of their phylogeny and taxonomy is controversial (e.g., Szczerbak, 1974; Guo *et al.*, 2011). Among the many controversies, the taxonomy of the *Eremias velox* complex is perhaps one of the most confusing. The distribution of this group ranges from the northern Caucasus across entire Central Asia eastward to China (Figure 1). Traditionally, the rapid racerunner was considered as a single species with three or four described subspecies (Szczerbak, 1974, 1975; Eremchenko and Panfilov, 1999). However, as noted by Rastegar-Pouyani (2009), it is increasingly common to accept *E. velox* as a clade in its entire range representing a species complex. The nominate form occurs in the larger parts of the distribution range. The Caspian subspecies *E. v. caucasia* occurs at the western part of the range. The

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subspecies *E. v. roborowskii* is endemic in Turpan-Hami Depression and Dunhuang Basin in northwestern China. Recently Eremchenko and Panfilov (1999) described the fourth subspecies, *E. v. borkini* from the highlands of Issyk-Kul lake depression in Kyrgyzstan.

Many recent taxonomic treatments reveal varied opinions as to the number of species. Some herpetologists recognized a single widespread species (e.g., Szczerbak, 2003; Ananjeva *et al.*, 2006; Sindaco and Jeremcenko, 2008), whereas others (Rastegar-Pouyani, 2009; Rastegar-Pouyani *et al.*, 2012) hypothesized the existence of at least four distinct species and three well-supported subspecies (Figure 2). Some of the differences of opinion result from the accumulation of mitochondrial DNA sequence information (Rastegar-Pouyani *et al.*, 2012). At present it seems reasonable that mtDNA sequence information supports the recognition of the Iranian populations of *E. velox* as three distinct species based on differences in distribution, morphology, habitat preference, and mtDNA sequence divergence (Figure 2).

The subspecific status of *E. v. roborowskii* is still controversial, although Szczerbak (1975) confirmed its status according to color, pattern and characters of pholidosis. Boulenger (1921) suggested that the morphological variation in the populations of *E. v. roborowskii* is not sufficient to support its subspecies status. The validity of this subspecies has not been recognized for a long time (Mertens and Wermuth, 1960). More recently, on the basis of multivariate morphological analyses, Wang *et al.* (2014) argued that there is no subspecies differentiation between populations from Turpan-Hami Depression and from Dzungarian Basin and Ily Valley in China. Thus, Wang *et al.* (2014) implied that *E. v. roborowskii* may be a synonym of nominate subspecies.

Establishing correct species/subspecies limits depends on adequate sampling of populations throughout the range. Given the previous studies, it might appear that taxonomic limits were clear. Rastegar-Pouyani (2009) made the first attempt to elucidate the phylogenetic relationships among the rapid racerunner in Iran and Central Asia on the basis of ISSR-PCR fingerprints data. The results were tentative due in part to limited taxonomic sampling. As shown by simulations and empirical studies, more taxa and data do affect the phylogenetic reconstruction (e.g., Pollock *et al.*, 2002; Zwickl and Hillis, 2002). Rastegar-Pouyani *et al.* (2012) studied patterns of mitochondrial DNA cytochrome *b* (cyt *b*) and 12S rRNA variation in 70 specimens from 13 geographically distant localities in Iran and Central Asia. Their results contradicted previous systematic hypotheses; revealed surprising relationships between Iranian and Central Asiaian lineages and uncovered novel, cryptic evolutionary lineages (i.e. new putative species). Moreover, they argued that the eastern and southern Kazakhstan populations correspond to the traditional *E. v. roborowskii*. However, Rastegar-Pouyani *et al.* (2012), who used most samples already analyzed by Rastegar-

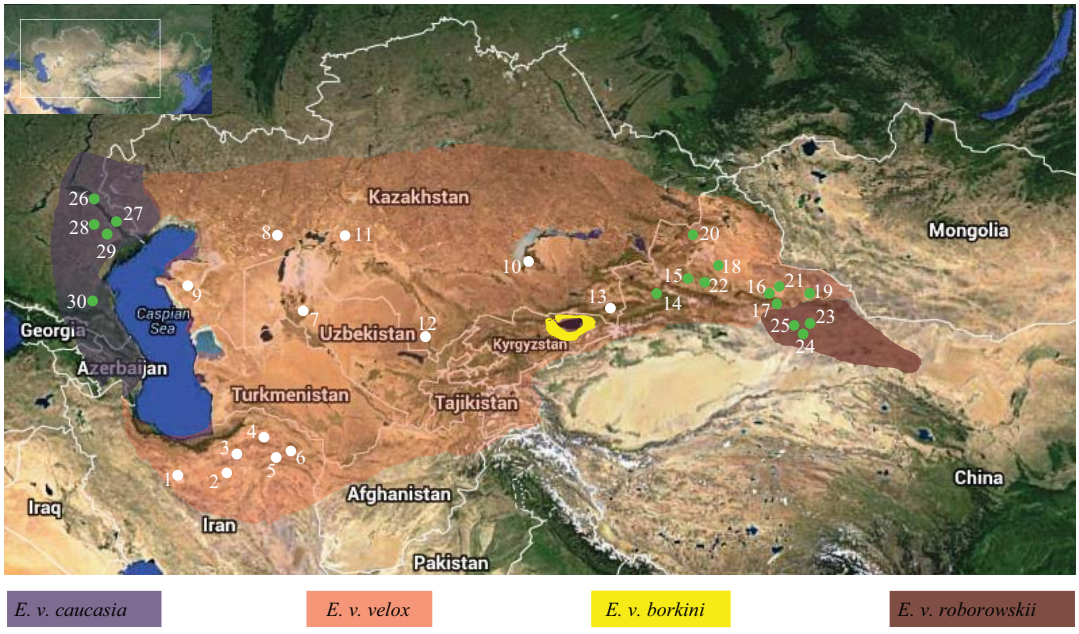


**Figure 1** Entire distribution range of *Eremias velox* complex showing traditional subspecies' range with different background color, and the sampling localities for different populations (numerical labels 14–30 with green dots), white dots with numbers 1–13 representing those from Rastegar-Pouyani *et al.*(2012). The locality number corresponds with that in Appendix A.

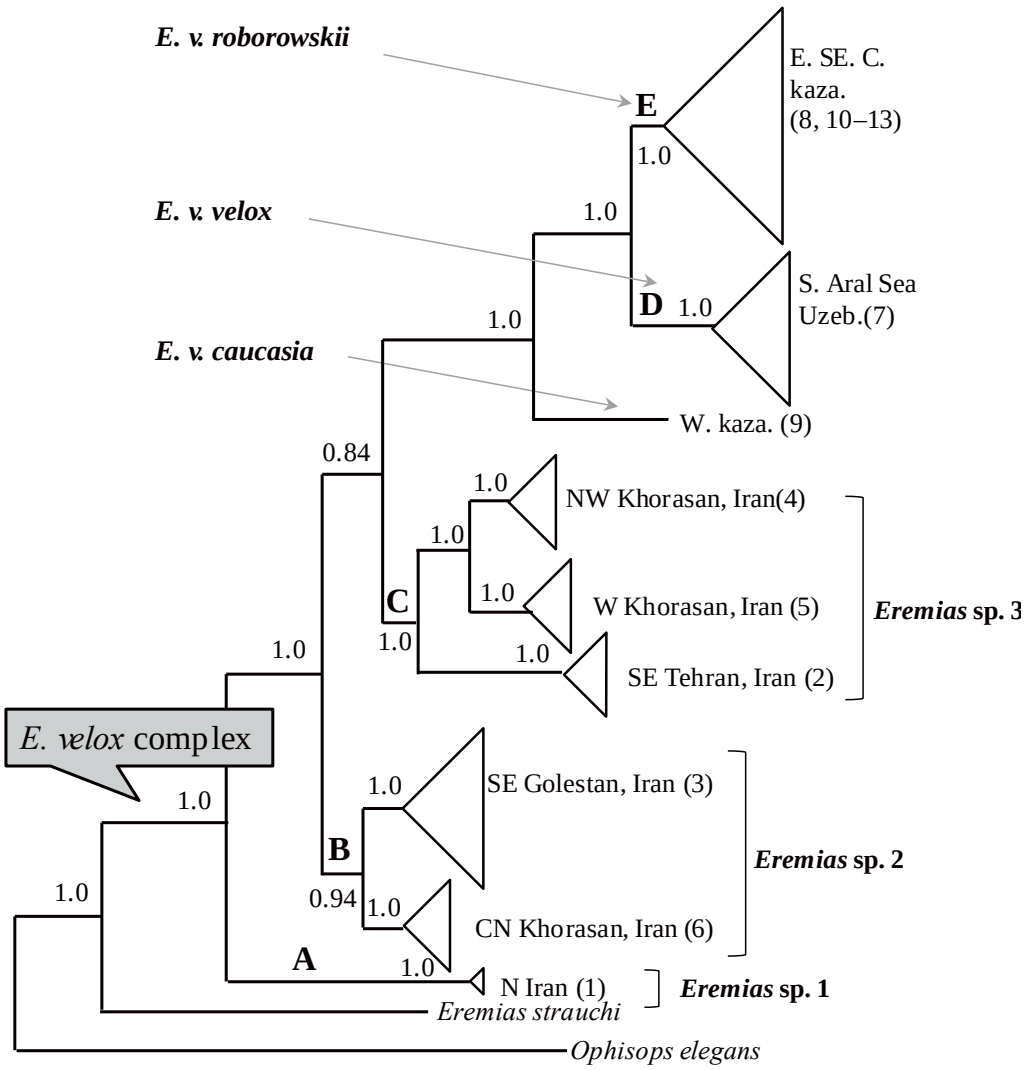


**Figure 2** Rastegar-Pouyani *et al*.'s (2012) phylogenetic hypothesis for populations of the *Eremias velox* complex from Iranian Plateau and Central Asia using partitioned Bayesian analyses of mtDNA data. A, B, C, D and E indicate the major clades. Values beside the node represent Bayesian posterior probabilities.

Pouyani (2009), were unable to sample adequately the eastern part of the taxon's range. There was no sample from China included in their work. In addition, Rastegar-Pouyani *et al.* (2012) speculated that *E. v roborowskii* occurs in western China, eastern Kazakhstan and Eastern Uzbekistan. This inappropriate interpretation may give confusing clues about the identity of this subspecies, resulting in incorrect inference of its phylogenetic placement. It appears likely that the inclusion of more rapid racerunner samples may get a better illustration of their phylogeographic structure, and may also provide valuable information for their taxonomy.

Thus, as an extension of the molecular phylogenetics of the rapid racerunner, an effort has been made to collect some specimens from northwestern China and Russia. We further determined the complete cyt *b* gene and 12S rRNA segment to gain a more comprehensive view on phylogeographic patterns in *E. velox* complex. This study specifically aims to: (1) re-evaluate the phylogeographic structure of *E. velox* complex, (2) test the phylogenetic affinities of the rapid racerunner populations in China, and (3) test the validity of the subspecific status of *E. v. roborowskii*. The hypothesis that *E. v. roborowskii* is valid particularly predicts that it will correspond with distinct, well-supported, and moderately divergent mtDNA haplotype clade.

## 2. Materials and Methods

**2.1 Taxon sampling** We collected a total of 87 samples representing natural populations at 17 localities. These included 57 samples from 12 localities in China, 30

samples from five localities in Russia, covering a large territory occupied by *E. velox* in the two regions (Appendix A, Figure 1). To ensure accuracy more than four representatives of each population were collected. However, only a single individual was collected from six sites due in part to sampling difficulties. Populations were assigned subspecific status based on location and phenotype. Due to logistical and legal obstacles to filed work, no sample of *E. velox borkini* from the highlands of Kyrgyzstan was available. Based on present knowledge of phylogenetic relationships among *Eremias* lizards, *E. argus* and *E. persica* were chosen as outgroup taxa (Guo *et al.*, 2011). All voucher specimens were deposited in the herpetological collections of Chengdu Institute of Biology, Chinese Academy of Sciences or the Department of Zoology, University of Guelph. Detailed information about all specimens and sequences used in this study is listed in Appendix A.

**2.2 DNA extraction, amplification, and sequencing** Genomic DNA was extracted from liver or tail muscle tissues stored in 95% ethanol following the protocol of Aljanabi and Martinez (1997). Complete sequence of the mitochondrial DNA cyt *b* gene was amplified using the primers L14919 and H16064 from Burbrink *et al.* (2000). A ~371bp fragment of 12S rRNA was amplified using the primers L1091 and H1478 from Kocher *et al.* (1989). Each of our 50 μl PCR reactions contained 25 μl of 2 × EasyTaq SuperMix, 0.4 μM of each primer, and 1–2 μl genomic DNA. The PCR protocol involved initial denaturation at 94°C for 180 s followed by 30 cycles of 94°C for 30 s, 43°C for 30 s (50–52°C for 12S rRNA), and elongation at 72°C for 90 s (60 s for 12S rRNA); and final extension at 72°C for 8 min. Negative controls were run for all amplifications. Amplified products were visualized on 0.8% agarose gels and purified using the DNA gel extraction kit (Omega Bio-Tek). The purified products were sequenced directly by Majorbio or Sangon (Shanghai) Co., Ltd., using the same primers as PCR for 12S rRNA, and L14919 from Burbrink *et al.* (2000) in combination with L14761 as well as H14892 from Zhao *et al.* (2011) for cyt *b*. All novel sequences are deposited in GenBank with accession numbers KF999318–KF999491.

**2.3 Sequence alignment and analyses** We have retrieved sequences of cyt *b* and 12S rRNA of 39 individuals from GenBank, which included sequences of 37 individuals published in Rastegar-Pouyani *et al.* (2012) and sequences of two individuals used as outgroup in this study (Appendix A). Sequences of cyt *b* were translated to amino acid sequences using MEGA5 (Tamura *et al.*, 2011) to verify the data. A total of 126 sequences of cyt *b* and 12S rRNA were first aligned using Clustal X 2.0 (Larkin *et al.*, 2007), respectively, with default gap penalties. Then the aligned matrice were re-checked visually.

Phylogenetic congruence of cyt *b* and 12S rRNA data sets were tested by the partition homogeneity test of Farris *et al.* (1995) with PAUP* 4.0b10 (Swofford, 2003). The partition homogeneity test supported the combination of the cyt *b* and 12S rRNA data sets ($P = 0.1$). Homogeneity of base frequencies was evaluated using Chi-square ($\chi^2$) tests implemented in PAUP*. Base composition at the third position exhibited no heterogeneity: the combined data, $\chi^2 = 16.66$, $df = 237$, $P = 1.00$; first position, $\chi^2 = 7.18$, $df = 237$, $P = 1.00$; second position, $\chi^2 = 2.02$, $df = 237$, $P = 1.00$; third position, $\chi^2 = 48.99$, $df = 237$, $P = 1.00$; and 12S rRNA, $\chi^2 = 12.11$, $df = 237$, $P = 1.00$. Given these results, a sensible approach to analyze these data phylogenetically would be to apply a time-reversible Markov model.

**2.4 Phylogenetic analyses** To estimate phylogenetic relationships, Bayesian inference (BI), maximum likelihood (ML), and maximum parsimony (MP) were implemented for the combined data. Each haplotype was treated as a taxon in the analyses. MP analyses with PAUPRat (Sikes and Lewis, 2001), based on Parsimony Ratchet (Nixon, 1999), were conducted using 1000 ratchets with 10 iterations per replicate. The shortest equally most parsimonious trees were combined to produce a strict consensus tree. Consistency (CI), retention (RI), and rescaled consistency (RC) indices were used to evaluate homoplasy in character data and overall support for optimal trees. Support for individual branches was evaluated using non-parametric bootstrapping (Felsenstein, 1985) in PAUP* with 1000 pseudoreplicates, TBR branch swapping, simple taxon addition with one tree held at each step, and a maximum of 100 trees saved per replicate in order to decrease the time needed to run large bootstrap replicates.

To infer relationships under BI we used MrBayes v3.2.2 (Ronquist *et al.*, 2012). We optimized several parameters prior to conducting our final analyses using the Markov Chain Monte Carlo (MCMC) method implemented in MrBayes, including partitioning strategy, models of molecular evolution, length of MCMC analyses. We assessed two alternative partitioning strategies: (1) a single partition and (2) a four-partition strategy that applied a single partition to each of the three codon positions of cyt *b* and a fourth partition for 12S

rRNA sequence.

The Akaike Information Criterion (AIC; Akaike, 1974), as implemented in MrModeltest v2.3 (Nylander, 2008), was used to select the appropriate model of sequence evolution for each partition. To compare the two alternative partitioning strategies, we conducted two independent analyses of each strategy in MrBayes. Each analysis consisted of three heated chains and one cold chain, with an incremental heating temperature of 0.02, and an exponential distribution with a rate parameter of 25 as the prior on branch lengths (Marshall, 2010). All analyses were run for 20 million generations with parameters and topologies sampled every 1000 generations. To ensure MCMC convergence, a burn-in period for all analyses was diagnosed in two ways: (1) by the average standard deviation of split frequencies (ASDSF; Lakner *et al.*, 2008) between two MCMC analyses run independently, with levels below 0.01 being considered indicative of convergence, and (2) by the potential scale reduction factor (PSRF; Gelman and Rubin, 1992) comparing the variance within and between runs, with levels approaching 1.0 as runs converge. To compare performance of the two partitioning strategies we calculated Bayes factors from the marginal likelihood scores estimated by the stepping-stone method (Xie *et al.*, 2011) in MrBayes 3.2.2. Comparison of alternative partitioning strategies strongly favored four partitions over one partitioning scheme (2Ln Bayes factor > 600). After selecting the optimal partitioning strategy, we conducted two analyses with different starting trees using the optimized parameters. We then used the *sumt* command in MrBayes to generate a consensus topology and associated Bayesian posterior probabilities (BPP) from a pooled post-burnin sampled from two analyses.

Partitioned maximum likelihood (ML) analyses were conducted in RAxMLHPC v7.0 (Stamatakis, 2006) on the combined dataset with the same partitioning strategy as for Bayesian inference. The more complex model (GTR + $I$ + $\Gamma$) was used for all subsets, and 100 replicate ML inferences were performed for each analysis. Each inference was initiated with a random starting tree and nodal support was assessed with 1000 bootstrap pseudoreplicates (Stamatakis *et al.*, 2008).

**2.5 Bayesian tests of topology hypotheses** Bayes factors were used to compare the unconstrained Bayesian tree topology to Bayesian trees with 'hard' constraints. Here we tested the following taxonomy and geography-based hypotheses to evaluate the taxonomic status within *E. velox* complex: (1) Are populations within the historically accepted distributions in northwestern China monophyletic? (2) Are the Iranian populations monophyletic? (3) Do Iranian populations belong to nominate subspecies? (4) Are populations of *E. v. roborowskii* and nominate subspecies monophyletic? We constrained the topology for each of the above putative groupings and performed a stepping stone run of 10 million generations (50 steps with convergence being reached in each step) from which we obtained a mean marginal likelihood estimate. Convergence was examined through diagnostic plots of standard deviation of the split frequencies and screening similarity in the two independent marginal likelihood estimates (Ronquist *et al.*, 2011). Each of these estimates were compared using Bayes factors (Kass and Raftery, 1995) to the marginal likelihood estimate obtained from the unconstrained MrBayes run of the same length. The stepping stone function (Xie *et al.*, 2011) implemented in MrBayes v3.2.2, offers an improved estimation of marginal likelihood over harmonic mean estimation.

## 3. Results

**3.1 Sequence characteristics** Two mitochondrial genes (cyt *b* and 12S rRNA) were obtained, yielding alignments of 1143 and 372 bp, respectively. 126 sequences (including two outgroup taxa) revealed 80 haplotypes. Alignment was unambiguous and 1 indel was inferred in 12S rRNA between outgroup and ingroup taxa. No premature stop codons were observed in cyt *b*, indicating that the obtained sequences were mitochondrial in origin and not nuclear pseudocopies. Average base composition is *A*: 27.55%, *C*: 30.69, *G*: 14.72%, *T*: 27.04%, when the outgroup taxa are combined. Nucleotide composition showed an anti-*G* bias and *A*+*T*-richness (54.59%), which is a characteristic of the mitochondrial genome. Variable and parsimony-informative characters are: 441 and 319 of 1143 (cyt *b*); 84 and 57 of 371 (12S rRNA).

A $\chi^2$ test at the 5% level of significance for differences in base frequencies showed that there was no base compositional heterogeneity among sequences, which is known to adversely affect phylogenetic inference (Jermiin *et al.*, 2004).

**3.2 Phylogenetic analyses** The heuristic search of the combined data resulted in 914 equally parsimonious trees of 1136 steps, with moderate CI (0.579) and high RI (0.887). These are presented as a majority-rule consensus tree where branches with bootstrap support lower than 50% are collapsed (data not shown). AIC analyses conducted with the aid of MrModeltest identified the

HKY + $I$ + $\Gamma$ model as the most appropriate model for partition of cyt *b* 1st codon position, and the GTR + $I$ + $\Gamma$ model for partitions of cyt *b* 2nd codon position, cyt *b* 3rd codon position, 12S rRNA or combined data. Bayesian analyses were conducted for 20 million generations, with a conservative burn-in of six million generations that provided dense sampling of the posterior distribution. The MP, ML and Bayesian trees differed in support values, but not in the compositions of the major lineages. The bootstrap support was also marked on the branches that receive such support in the Bayesian tree (Figure 3). The phylogenetic trees recovered the monophyly of the *E. velox* complex with high support values. As shown in Figure 3, seven major clades connected deep in the complex phylogenetic history were identified in all reconstructions with strong posterior probabilities and bootstrap values, which generally correspond well to geographical regions within the distribution range. These clades were designated I, II, III, IV, V, VI and VII in Figure 3. The subdivision and phylogenetic positions of the Iran populations (I, II, III) are completely identical with the clades A, B, C of Rastegar-Pouyani *et al*. (2012), respectively. The basal split in the complex is between the northern Iranian population (clade I) and a highly supported clade of the other populations from Iran, Central Asia, China and Russia. However, clade IV, corresponding to *E. v. roborowskii*, covers the populations from Turpan-Hami Depression, and forms the sister taxon to the other populations excluding those from Iran. The clade V, corresponding to *E. v. caucasia*, covers populations from North-eastern Russian Caucasus and Western Kazakhstan. The clade VI is composed of populations from Uzbekistan, conventionally assigned to nominate subspecies. The clade VII covers populations from Eastern Kazakhstan and Dzungarian Basin as well as Ily Valley in China, which corresponds to nominate subspecies. Overall, the notable differences between Rastegar-Pouyani *et al.* (2012) and our results presented here lie in the phylogenetic placement of *E. v. roborowskii.*

**3.3 Bayesian tests of topology hypotheses** We compared the marginal likelihood estimates of topologically constrained and unconstrained run of MrBayes for possible monophyletic group within *E. velox* complex (Table 1). In all cases, there was very strong (Bayes factor > 100) evidence against the constrained topologies. Thus, four alternative phylogenetic hypotheses were significantly rejected, such as a monophyletic group of Chinese rapid racerunner populations, monophyly of Iranian populations, assignment of Iranian populations to nominate subspecies, and assignment of *E. v. roborowskii* to nominate subspecies.

## 4. Discussion

This study provided strong support for the monophyly of *E. velox*, consistent with the results of Guo *et al.* (2011) and Rastegar-Pouyani *et al.* (2012), and thus providing a firm basis for future testing of alternative biogeographic models. The Iranian populations were recovered here as a paraphyletic assemblage, representing three distinct, biogeographically discrete, well-supported clades that are not each other's closet relatives. This is in accord with the results of Rastegar-Pouyani *et al.* (2012).

The morphological differences (Rastegar-Pouyani *et al.*, 2012), allopatric ranges and substantial genetic divergence in the mtDNA bear evidence of long standing isolation between several populations. The haplotype clades associated with each lineage on Iranian Plateau are differentiated by mean uncorrected levels of sequence divergence that often exceed 10% (Table 2). This degree of divergence suggests that evolutionary separation of the three clades occurred millions of years ago, likely sometime during the Miocene based on a molecular clock calibration of 1.25% pairwise divergence per million years for mtDNA within the *E. velox* complex (Rastegar-Pouyani *et al.*, 2012). Concordance between strongly differentiated mtDNA haplotype clades and phenotypic

**Table 1** Bayes factors of stepping-stone-based estimates of marginal likelihood for comparisons of alternative phylogenetic hypotheses. 2ln Bayes factors ≥10 are considered very strongly different (Kass and Raftery, 1995), indicating evidence against alternative hypotheses.

| Alternative phylogenetic hypotheses | | Description of constraint | Ln Marginal likelihood | | 2Ln Bayes factor |
|---|---|---|---|---|---|
| | | | Ln*L*: unconstrained | Ln*L*: constrained | |
| H1 | Monophyly of rapid racerunner populations in China | All samples from China | –8022.39 | –8121.34 | 197.9 |
| H2 | Monophyly of rapid racerunner populations in Iran | All samples from Iran: clade I, II, III | –8022.39 | –8076.55 | 108.32 |
| H3 | Nominate subspecies affinity of Iranian rapid racerunner populations | clade I, II, III, VI, VII | –8022.39 | –8082.49 | 120.2 |
| H4 | Monophyly of *E. v. velox* and *E. v. roborowskii* | clade IV, VI, VII | –8022.39 | –8090.19 | 135.6 |

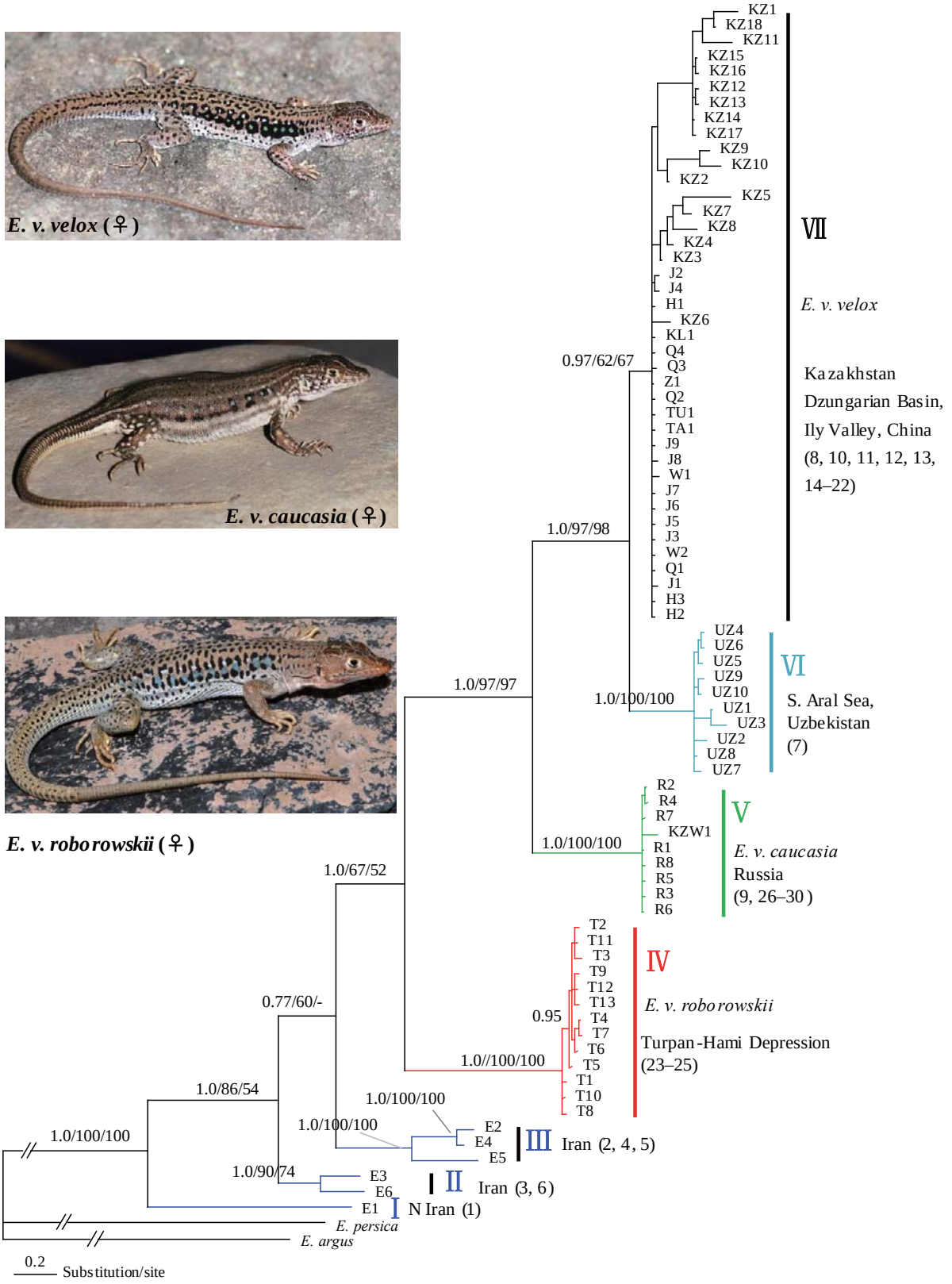


**Figure 3** Hypothesized relationships of *Eremias velox* complex included in this study, illustrated by the majority-rule consensus tree resulting from partitioned Bayesian analyses. Bayesian posterior probabilities, maximum parsimony and likelihood bootstrap values are shown. Nodes supported by ≥ 0.95 Bayesian PP and ≥ 70% BP were considered highly supported. Terminals are labeled with haplotypes. Roman alphabet labels correspond to clades referred to the Results and Discussion. Representative photographs of female adults show the dorsal and lateral differentiation among three subspecies.

**Table 2** Genetic distances within and between outgroup species and *E. velox* complex included in this study. Mean uncorrected (*p*) distances are below diagonal; distances calculated using MEGA's Maximum Composite Likelihood model with rate variation among sites defined by the gamma shape parameter (0.2366) are above the diagonal. Mean maximum likelihood divergences within each species or subspecies are in the diagonal with bold text.

| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | *E.argus* | **NA** | 0.419 | 0.483 | 0.442 | 0.443 | 0.454 | 0.453 | 0.471 | 0.433 |
| 2 | *E. persica* | 0.161 | **NA** | 0.416 | 0.442 | 0.489 | 0.462 | 0.441 | 0.433 | 0.401 |
| 3 | clade I | 0.172 | 0.158 | **NA** | 0.173 | 0.251 | 0.267 | 0.272 | 0.325 | 0.299 |
| 4 | clade II | 0.164 | 0.159 | 0.099 | **0.03** | 0.092 | 0.145 | 0.152 | 0.171 | 0.164 |
| 5 | clade III | 0.165 | 0.17 | 0.122 | 0.066 | **0.033** | 0.14 | 0.167 | 0.186 | 0.17 |
| 6 | clade IV | 0.17 | 0.165 | 0.128 | 0.092 | 0.089 | **0.003** | 0.152 | 0.17 | 0.153 |
| 7 | clade V | 0.168 | 0.161 | 0.129 | 0.095 | 0.101 | 0.094 | **0.002** | 0.111 | 0.093 |
| 8 | clade VI | 0.166 | 0.154 | 0.14 | 0.099 | 0.105 | 0.098 | 0.075 | **0.006** | 0.04 |
| 9 | clade VII | 0.162 | 0.152 | 0.135 | 0.097 | 0.1 | 0.093 | 0.067 | 0.033 | **0.012** |

variation (Rastegar-Pouyani *et al.*, 2012) supports the hypothesis that each of the three independently evolving lineages from Iranian Plateau deserves recognition at the species-level under the general lineage concept of species (de Queiroz, 1999; 2007) and associated operational species-delimitation criteria (Wiens and Penkrot, 2002).

Rastegar-Pouyani *et al.* (2012) demonstrated the basal positions of the Iranian clades for the first time and considered them three distinct species. However, their inference of the phylogenetic placement of *E. v. roborowskii* was misleading. In support of Rastegar-Pouyani *et al.* (2012), our results confirm the basal position of the Iranian clades and statistically reject the placement of Iranian clades into the nominate subspecies from Central Asia. Surprisingly, we find that *E. v. roborowskii* is the sister taxon to *E. velox caucasia* plus *E. v. velox*, i.e., other populations from Central Asia. Our results clearly confirm that all of the major clades inferred from mitochondrial haplotypes are concordant with geographical regions. Iranian clades at the basal position of the phylogenetic tree may imply that *E. velox* originated from the Iranian Plateau and invaded Central Asia as Rastegar-Pouyani *et al.* (2012) suggested. If we take this scenario as a reliable explanation for the origin and evolution of *E. velox*, we consider the clade IV (*E. v. roborowskii*) as first split from entire Central Asian populations, resulting from the barrier effect of Tianshan Mountains with their dramatic uplift since Tertiary (Macey *et al.*, 1999; Guo and Wang, 2007; Zhang *et al.*, 2008; Melville *et al.*, 2009; Solovyeva, 2013). Geographically, with the uplift of Tianshan Mountains, Turpan-Hami Depression and Dzungarian Basin were blocked from each other, resulting in a historical vicariant pattern and allopatric distribution between *E. v. roborowskii* and *E. v. velox* as well as *E. v. caucasia*. Wang *et al.* (2014) failed to recognize the validity of *E. v. roborowskii* due to a lack of understanding the discrete characters between *E. v. velox* and *E. v. roborowskii*, including dorsal pattern and lateral color pattern. In general, the adults of *E. v. roborowskii* have a dorsal pattern of irregular dark spots, with rows of bright blue-edged, dark eye-like spots on the lateral sides (Zhao, 1999; Szczerbak, 2003).

In addition, clade V, corresponding to traditional *E. velox caucasia*, forms the sister taxon to Central Asian clades, in accord with the results of Dolotovskaya *et al.*(2007) inferred from ~ 1800 bp mtDNA of cyt *b*, COI and 16S rRNA . Rastegar-Pouyani *et al.* (2012) speculated that a single specimen from Western Kazakhstan belongs to *E. velox caucasia*. The affinity of that single specimen was corroborated in this study for there to be strong support for its nesting inside clade V. The Central Asian clades, corresponding to conventional nominate subspecies, comprise clades VI and VII. The patterns of mtDNA variation support a sister relationship between clade VI and clade VII. Clade VI comprises samples from Uzbekistan, whereas clade VII consists of populations from Kazakhstan and Dzungarian Basin and Ily River Valley, Xinjiang, China. Judging from the type locality of the nominate form of *E. velox*, Inderskija mountain in Kazakhstan, we consider clade VII as nominate subspecies. Szczerbak (1975) examined the relationships of *E. v. roborowskii* from Ssatschsheu (Dunhuang Basin, Gansu, China) and Lukuchun (Shanshan, Xinjiang, China) with a population from Dzungarian Basin. He found that the samples from Dzungarian Basin stood an intermediate position between the subspecies *E. v. roborowskii* and nominative form. On the one hand, judging from pholidosis, the population from Dzungarian Basin is closer to *E. v. roborowskii* than to the populations from Balkhash in Kazakhstan. On the other hand, the dorsal and lateral pattern of the population from Dzungarian Basin is closer to that of the latter, i.e. nominate form.

Interestingly, Szczerbak (1975) referred to the population from Dzungarian Basin as nominative form. Our results statistically support that the rapid racerunner populations in Dzungarian Basin cluster with those from Kazakhstan. In fact, biogeographic connections to the Dzungarian Basin are to the northwest, with a number of passes leading to Kazakhstan. The Dzungarian Gate is the main pass through the western ranges, leading to Lake Alaköl and Lake Balkhash in Kazakhstan. There are a number of taxa that have similar distribution pattern, including *Phrynocephalus melanurus* (Melville *et al.*, 2009), *P. heliocopus* (Solovyeva *et al.*, 2011), and *Eremias arguta* (Szczerbak, 2003).

Traditionally, the subspecies category is used to diagnose geographically distinct populations that were thought to be in the early stages of speciation (Mayr, 1963; Mayr and Ashlock, 1991). Despite some criticisms surrounding the concept of subspecies (e.g., Burbrink *et al.*, 2000; Patten and Unitt, 2002; Zink, 2004), recent studies in which researchers used multiple criteria (e.g., morphological, behavioral, and genetic characters) have confirmed that many subspecies are evolutionarily definable entities (e.g., Gavin *et al*., 1999; Phillimore and Owens, 2006; Guo *et al.*, 2012). In Central Asia, the clinal and discrete variation among *E. velox* populations from different regions have been documented (Szczerbak, 1975; Chirikova, 2004). As noted by Szczerbak (1975), the most deviating populations of *E. velox* were found in Fergana Valley and neighborhood of Badkhyz desert, reaching subspecies level. Chirikova (2004) studied geographic variations of *E. velox* populations from Kazakhstan and Uzbekistan. She found some variations in phenotypical traits between populations from the two regions. In contrast with the nominate form in Kazakhstan, populations from the southern Aral Sea region in Uzbekistan have very light elongated spots on the middle of the back in adults. Even some individuals from the southern Aral Sea region lack this kind of dorsal pattern completely. Our results demonstrate that the populations in southern Aral Sea region of Uzbekistan correspond with distinct, well-supported, and moderately divergent mtDNA haplotype clade (clade VI) . The mean uncorrected level of sequence divergence between clades VI and VII falls within 3.3%. In support of Chirikova (2004), we recommend that the populations of rapid racerunner from southern Aral Sea region of Uzbekistan be assigned to a novel subspecies, which will facilitate an effective short-cut for estimating patterns of intraspecific genetic diversity of *E. velox*.

## 5. Conclusions

The mtDNA data confirms the subspecific status for three of the four morphology-defined subspecies: *E. v. roborowskii*, *E. v. caucasia*, *E. v. velox* (sensu stricto). Specifically, we corroborate that there are two subspecies occurring in China, i.e., *E. v. velox* and *E. v. roborowskii*. We recommend a novel subspecies for the phenotypically and genetically distinct populations in Uzbekistan previously referred to as *E. v. velox*. We also support species-level recognition for each of the three independently evolving lineages from Iranian Plateau traditionally assigned to *E. v. velox*. We do so recognizing that more comprehensive geographic sampling and investigation of additional molecular markers are required to clarify hypothesized species/subspecies boundaries, taxonomic status and evolutionary history of the *E. velox* complex.

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## References

**Akaike H.** 1974. A new look at the statistical model identification. IEEE Trans Automat Contr, 19: 716–723

**Aljanabi S. M., Martinez I.** 1997. Universal and rapid salt-extraction of high quality genomic DNA for PCR-based techniques. Nucl Acids Res, 25: 4692–4693

**Ananjeva N. B., Borkin L., Darevsky I. S., Orlov N. L.** 1998. Amphibians and Reptilies. Encyclopedia of the Nature of Russia (Field Guide of Amphibians and Reptiles of Russia and Adjacent Countries). Moscow: ABF Publishing Company, 574 pp (In Russian)

**Ananjeva N. B., Orlov N. L., Khalikov R. G., Darevsky I. S., Ryabov I. S., Barabanov A. V.** 2006. The Reptiles of North Eurasia: Taxonomic Diversity, Distribution, Conservation Status. Bulgaria: Pensoft Publishers, 250 pp

**Boulenger G. A.** 1921. Monograph of the Lacertidae, Vol. II. London: Trustees of the British Museum of Natural History, VIII + 451 pp

**Burbrink F. T., Lawson R., Slowinski J. B.** 2000. Mitochondrial DNA phylogeography of the polytypic North American rat snake (*Elaphe obsoleta*): A critique of the subspecies concept. Evolution, 54: 2107–2118

**Chirikova M. A.** 2004. Variability of *Eremias velox* Pallas, 1771 (Reptilia, Sauria) from Kazakhstan. Selevinia, 24–34 (In Russian with English abstract)

**de Queiroz K.** 1998. The general lineage concept of species, species criteria, and the process of speciation: a conceptual unification and terminological recommendations. In Howard D. J., Berlocher S. H. (Eds), Endless Forms: Species and Speciation. Oxford: Oxford University Press, 57–75

**de Queiroz K.** 2007. Species concepts and species delimitation. Syst Biol, 56: 879–886

**Dolotovskaya S. I., Chirikova M. A., Solovyeva E. N., Poyarkov N. A., Wan L., Orlova V. F.** 2007. Molecular and morphological differentiation of rapid racerunner *Eremias velox* (Lacertidae) with comments on taxonomy and biogeography of Middle-Asian racerunners. Abstracts of 14th European Congress of Herpetology and SEH Ordinary General Meeting, Porto, Portugal, 203

**Eremchenko V., Panfilov A.** 1999. Taxonomic position and geographic relations of a lacertid lizard *Eremias velox* from the Issyk-Kul lake depression, Tien Shan Mountains, Kyrgyzstan. Science and New Technology, 99: 119–125

**Felsenstein J. P.** 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution, 39: 783–791

**Frankham R., Ballou J. D., Briscoe D. A.** 2002. Resolving taxonomic uncertainties and defining management units. In Frankham R., Ballou J. D., Briscoe D. A. (Eds), Introduction to Conservation Genetics. Cambridge, UK: Cambridge University Press, 365–392

**Gavin T. A., Sherman P. W., Yensen E., May B.** 1999. Population genetic structure of the northern Idaho ground squirrel (*Spermophilus brunneus brunneus*). J Mammal, 80: 156–168

**Guo X., Dai X., Chen D., Papenfuss T. J., Ananjeva N. B., Melnikov D. A., Wang Y.** 2011. Phylogeny and divergence times of some racerunner lizards (Lacertidae: *Eremias*) inferred from mitochondrial 16S rRNA gene segments. Mol Phylogenet Evol, 61: 400–412

**Guo X., Liu L., Wang Y.** 2012. Phylogeography of the *Phrynocephalus vlangalii* species complex in the upper reaches of the Yellow River inferred from mtDNA ND4-tRNA$^{LEU}$ segments. Asian Herpetol Res, 3: 52–68

**Guo X., Wang Y.** 2007. Partitioned Bayesian analyses, dispersal-vicariance analysis, and the biogeography of Chinese toad-headed lizards (Agamidae: *Phrynocephalus*): A re-evaluation. Mol Phylogenet Evol, 45: 643–662

**Jermiin L.**, **Ho S. Y.**, **Ababneh F.**, **Robinson J.**, **Larkum A. W.** 2004. The biasing effect of compositional heterogeneity on phylogenetic estimates may be underestimated. Syst Biol, 53: 638–643

**Kass R. E., Raftery A. E.** 1995. Bayes factors. J Am Statist Assoc, 90: 773–795

**Kocher T. D., Thomas W. K., Meyer A., Edwards S. V., Pääbo S., Villablanca F. X., Wilson A. C.** 1989. Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. Proc Natl Acad Sci, 86: 6196–6200

**Larkin M. A., Blackshields G., Brown N. P., Chenna R., McGettigan P. A., McWilliam H., Valentin F., Wallace I. M., Wilm A., Lopez R., Thomson J. D., Gibson T. J., Higgins D. G.** 2007. Clustal W and Clustal X version 2.0. Bioinformatics, 23: 2947–2948

**Macey J. R., Wang Y., Ananjeva N. B., Larson A., Papenfuss T. J.** 1999. Vicariant patterns of fragmentation among gekkonid lizards of the genus *Teratoscincus* produced by Indian collision: a molecular phylogenetic perspective and an area cladogram for Central Asia. Mol Phylogenet Evol, 12: 320–332

**Marshall D. C.** 2010. Cryptic failure of partitioned Bayesian phylogenetic analyses: Lost in the land of long trees. Syst Biol, 59: 108–117

**Mayr E.** 1963. Animal Species and Evolution. Cambridge, Massachusetts: Belknap Press, XVI + 797 pp

**Mayr E., Ashlock P. D.** 1991. Principles of Systematic Zoology, second edition. New York: McGraw-Hill, XX + 475 pp

**Melville J., Hale J., Mantziou G., Ananjeva N. B., Milto K., Clemann N.** 2009. Historical biogeography, phylogenetic relationships and intraspecific diversity of agamids lizards in the Central Asian deserts of Kazakhstan and Uzbekistan. Mol Phylogenet Evol, 53: 99–122

**Mertens R., Wermuth H.** 1960. Die Amphibien und Reptilien Europas. Verlag Waldemar Kramer, Frankfurt am Main. XII + 264 pp

**Nixon K. C.** 1999. The Parsimony Ratchet, a new method for rapid parsimony analysis. Cladistics, 15: 407–414

**Nylander J. A. A.** 2008. MrModeltest 2.3. Program distributed by the author. Evolutionary Biology Centre, Uppsala University

**Patten M. A., Unitt P.** 2002. Diagnosability versus mean differences of sage sparrow subspecies. Auk, 119: 26–35

**Phillimore A. B., Owens I. P. F.** 2006. Are subspecies useful in evolutionary and conservation biology? Proc R Soc B, 273: 1049–1053

**Pollock D. D., Zwickl D. J., Mccguire J. A., Hillis D. M.** 2002. Increased taxon sampling is advantageous for phylogenetic inference. Syst Biol, 51: 664–671

**Rastegar-Pouyani E.** 2009. The phylogeny of the *Eremias velox* complex of the Iranian Plateau and Central Asia (Reptilia, Lacertidae): Molecular evidence from ISSR-PCR fingerprints. Iranian J Animal Biosystemat, 5: 33–46

**Rastegar-Pouyani E., Kazemi Noureini S., Rastegar-Pouyani N., Joger U., Wink M.** 2010. Molecular phylogeny and biogeography of the *Eremias persica* complex of the Iranian Plateau (Reptilia: Lacertidae) based on sequences of the mtDNA. Zool J Linn Soc, 158: 641–660

**Rastegar-Pouyani E., Kazemi Noureini S., Rastegar-Pouyani N., Joge, U., Win, M.** 2012. Molecular phylogeny and intraspecific differentiation of the *Eremias velox* complex of the Iranian Plateau and Central Asia (Sauria, Lacertidae). J Zool Systemat Evol Res, 50: 220–229

**Ronquist F., Huelsenbeck J. P., Teslenko M.** 2011. Draft MrBayes version 3.2 manual: Tutorial and model summaries

**Ronquist F., Teslenko M., van der Mark P., Ayres D. L., Darling A., Höhna S., Larget B., Liu L., Suchard M. A., Huelsenbeck**

**J. P.** 2012. MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. Syst Biol, 61: 539–42

**Sikes D. S., Lewis P. O.** 2001. PAUPRat: PAUP* implementation of the parsimony ratchet. Beta software, version 1

**Sindaco R., Jeremcenko V. K.** 2008. The Reptiles of the Western Palearctic. 1. Annotated Checklist and Distributional Atlas of the Turtles, Crocodiles, Amphisbaenians and Lizards of Europe, North Africa, Middle East and Central Asia. Latina, Italy: Edizioni Belvedere, 579 pp

**Solovyeva E. N., Poyarkov N. A., Dunaev E. A., Duysebayeva T. N., Bannikova A. A.** 2011. Molecular differentiation and taxonomy of the sunwatcher toad-headed agama species complex *Phrynocephalus* superspecies *helioscopus* (Pallas 1771) (Reptilia: Agamidae). Russ J Genet, 47: 842–856

Solovyeva E. N. 2013. Genetic variability and the phylogeny of the genus *Phrynocephalus* (Reptilia: Agamidae). Ph.D. Thesis. Lomonosov Moscow State University, Moscow, Russia (In Russian)

**Stamatakis A.** 2006. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics, 22: 2688–2690

**Stamatakis A., Hoover P., Rougemont J.** 2008. A rapid bootstrap algorithm for the RAxML Web Servers. Syst Biol, 57: 758–771

**Swofford D. L**. 2003. PAUP*. Phylogenetic Analysis Using Parsimony (* and Other Methods), Version 4. Sunderland, MA: Sinauer

**Szcerbak N. N.** 1974. The Palearctic Desert Lizards *(Eremias*). Kiev: Naukova Dumka, 296 pp (In Russian)

**Szcerbak N. N.** 1975. Geographical variability and intraspecies taxonomy of *Eremias velox* Pall., 1771 (Reptilia, Sauria). Vestnik Zool, 6: 24–33 (In Russian with English abstract)

**Szczerbak N. N.** 2003. Guide to the Reptiles of the Eastern Palearctic. Malabar, Florida: Krieger Publishing Company, 260 pp

**Tamura K., Peterson D., Peterson N., Stecher G., Nei M., Kumar S.** 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol Biol Evol, 28: 2731–2739

**Wang Y., Zhou L., Shi L.** 2014. Scalation varaiton and subspecies classification status of rapid racerunner (*Eremias velox*) in Xinjiang. Sichuan J Zool, 33: 13–16 (In Chinese with English abstract)

**Wiens J. J., Penkrot T. A.** 2002. Delimiting species using DNA and morphological variation and discordant species limits in spiny lizards (*Sceloporus*). Sys Biol, 51: 69–91

**Xie W., Lewis P. O., Fan Y., Kuo L., Chen M.-H.** 2011. Improving marginal likelihood estimation for Bayesian phylogenetic model selection. Syst Biol, 60: 150–160

**Zhang Y., Stock M., Zhang P., Wang X., Zhou H., Qu L.** 2008. Phylogeography of a widespread terrestrial vertebrate in a barely studied Palearctic region: green toads (*Bufo viridis* subgroups) indicate glacial refugia in eastern Central Asia. Genetica, 134: 353–365

**Zhao K.-T., 1999.** Lacertidae. In Zhao E.-M., Zhao K.-T., Zhou K.-Y. (Eds), Fauna 3 Sinica, Reptilia (Squamata: Lacertilia), Vol. 2. Beijing: Science Press, 219–242 (In Chinese)

**Zhao Q., Liu H. X., Luo L. G., Ji X.** 2011. Comparative population genetics and phylogeography of two lacertid lizards (*Eremias argus* and *E. brenchleyi*) from China. Mol Phylogenet Evol, 58: 478–491

**Zink R. M.** 2004. The role of subspecies in obscuring avian biological diversity and misleading conservation policy. Proc R Soc B, 271: 561–564

**Zwickl D. J., Hillis D. M.** 2002. Increased taxon sampling greatly reduces phylogenetic error. Syst Biol 51, 588–598

**Appendix A** List of analyzed specimens, their geographical origin and the GenBank accession number.

| Taxon | Locality No. | Geographical origin | Latitude, longitude | Sample No. | Haplotype type | Accession number | | Reference |
|---|---|---|---|---|---|---|---|---|
| | | | | | | cyt *b* | 12S rRNA | |
| *E. v. velox* | 14 | Huocheng, Xinjiang, China | 44.02°N, 80.81°E | Guo475 | H1 | KF999318 | KF999405 | This study |
| *E. v. velox* | 14 | Huocheng, Xinjiang, China | 44.02°N, 80.81°E | Guo476 | Z1 | KF999319 | KF999406 | This study |
| *E. v. velox* | 14 | Huocheng, Xinjiang, China | 44.02°N, 80.81°E | Guo478 | Z1 | KF999320 | KF999407 | This study |
| *E. v. velox* | 14 | Huocheng, Xinjiang, China | 44.02°N, 80.81°E | Guo479 | Z1 | KF999321 | KF999408 | This study |
| *E. v. velox* | 14 | Huocheng, Xinjiang, China | 44.02°N, 80.81°E | Guo481 | Z1 | KF999321 | KF999409 | This study |
| *E. v. velox* | 14 | Huocheng, Xinjiang, China | 44.02°N, 80.81°E | Guo483 | H2 | KF999323 | KF999410 | This study |
| *E. v. velox* | 14 | Huocheng, Xinjiang, China | 44.02°N, 80.81°E | Guo484 | H3 | KF999324 | KF999411 | This study |
| *E. v. velox* | 14 | Huocheng, Xinjiang, China | 44.02°N, 80.81°E | Guo485 | Z1 | KF999325 | KF999412 | This study |
| *E. v. velox* | 14 | Huocheng, Xinjiang, China | 44.02°N, 80.81°E | QY1015 | Z1 | KF999396 | KF999483 | This study |
| *E. v. velox* | 14 | Huocheng, Xinjiang, China | 44.02°N, 80.81°E | QY1016 | TU1 | KF999397 | KF999484 | This study |
| *E. v. velox* | 14 | Huocheng, Xinjiang, China | 44.02°N, 80.81°E | QY1017 | Z1 | KF999398 | KF999485 | This study |
| *E. v. velox* | 15 | Jinghe, Xinjiang, China | 44.55°N, 82.59°E | Guo520 | Z1 | KF999326 | KF999413 | This study |
| *E. v. velox* | 15 | Jinghe, Xinjiang, China | 44.55°N, 82.59°E | Guo521 | Z1 | KF999327 | KF999414 | This study |
| *E. v. velox* | 15 | Jinghe, Xinjiang, China | 44.55°N, 82.59°E | Guo522 | J1 | KF999328 | KF999415 | This study |
| *E. v. velox* | 15 | Jinghe, Xinjiang, China | 44.55°N, 82.59°E | Guo523 | Z1 | KF999329 | KF999416 | This study |
| *E. v. velox* | 15 | Jinghe, Xinjiang, China | 44.55°N, 82.59°E | Guo524 | Z1 | KF999360 | KF999447 | This study |
| *E. v. velox* | 15 | Jinghe, Xinjiang, China | 44.55°N, 82.59°E | Guo525 | J2 | KF999361 | KF999448 | This study |
| *E. v. velox* | 15 | Jinghe, Xinjiang, China | 44.55°N, 82.59°E | Guo526 | J3 | KF999362 | KF999449 | This study |
| *E. v. velox* | 15 | Jinghe, Xinjiang, China | 44.55°N, 82.59°E | Guo527 | J4 | KF999363 | KF999450 | This study |
| *E. v. velox* | 15 | Jinghe, Xinjiang, China | 44.55°N, 82.59°E | Guo528 | J5 | KF999364 | KF999451 | This study |
| *E. v. velox* | 15 | Jinghe, Xinjiang, China | 44.55°N, 82.59°E | Guo529 | Z1 | KF999365 | KF999452 | This study |
| *E. v. velox* | 15 | Jinghe, Xinjiang, China | 44.55°N, 82.59°E | Guo530 | Z1 | KF999366 | KF999453 | This study |
| *E. v. velox* | 15 | Jinghe, Xinjiang, China | 44.55°N, 82.59°E | Guo531 | J6 | KF999367 | KF999454 | This study |
| *E. v. velox* | 15 | Jinghe, Xinjiang, China | 44.55°N, 82.59°E | Guo532 | Z1 | KF999368 | KF999455 | This study |
| *E. v. velox* | 15 | Jinghe, Xinjiang, China | 44.55°N, 82.59°E | Guo533 | Z1 | KF999369 | KF999456 | This study |
| *E. v. velox* | 15 | Jinghe, Xinjiang, China | 44.55°N, 82.59°E | Guo534 | J7 | KF999370 | KF999457 | This study |
| *E. v. velox* | 15 | Jinghe, Xinjiang, China | 44.55°N, 82.59°E | Guo535 | Z1 | KF999371 | KF999458 | This study |
| *E. v. velox* | 15 | Jinghe, Xinjiang, China | 44.55°N, 82.59°E | Guo536 | J8 | KF999388 | KF999475 | This study |
| *E. v. velox* | 15 | Jinghe, Xinjiang, China | 44.55°N, 82.59°E | Guo537 | J9 | KF999389 | KF999476 | This study |
| *E. v. velox* | 15 | Jinghe, Xinjiang, China | 44.55°N, 82.59°E | Guo538 | Z1 | KF999390 | KF999477 | This study |
| *E. v. velox* | 16 | Fukang, Xinjiang, China | 44.41°N, 87.86°E | WGXG08103 | Z1 | KF999330 | KF999417 | This study |
| *E. v. velox* | 17 | Dabancheng, Xinjiang, China | 43.34°N, 88.22°E | WGXG08525 | Z1 | KF999384 | KF999471 | This study |
| *E. v. velox* | 17 | Dabancheng, Xinjiang, China | 43.34°N, 88.22°E | WGXG08526 | W1 | KF999385 | KF999472 | This study |
| *E. v. velox* | 17 | Dabancheng, Xinjiang, China | 43.37°N, 88.14°E | WGXG08536 | Z1 | KF999386 | KF999473 | This study |
| *E. v. velox* | 17 | Dabancheng, Xinjiang, China | 43.37°N, 88.14°E | WGXG08537 | Z1 | KF999387 | KF999474 | This study |
| *E. v. velox* | 17 | Dabancheng, Xinjiang, China | 43.37°N, 88.14°E | WGXG08538 | W2 | KF999359 | KF999446 | This study |
| *E. v. velox* | 17 | Dabancheng, Xinjiang, China | 43.37°N, 88.14°E | WGXG08539 | W2 | KF999358 | KF999445 | This study |
| *E. v. velox* | 18 | Karamay, Xinjiang, China | 45.27°N, 85.03°E | GP07080433 | KL1 | KF999404 | KF999491 | This study |
| *E. v. velox* | 19 | Qitai , Xinjiang, China | 44.29°N, 90.11°E | WGXG08116 | Q1 | KF999331 | KF999418 | This study |
| *E. v. velox* | 19 | Qitai , Xinjiang, China | 44.29°N, 90.11°E | WGXG08118 | Q2 | KF999399 | KF999486 | This study |
| *E. v. velox* | 19 | Qitai , Xinjiang, China | 44.29°N, 90.11°E | WGXG08119 | Z1 | KF999400 | KF999487 | This study |
| *E. v. velox* | 19 | Qitai , Xinjiang, China | 44.29°N, 90.11°E | WGXG08120 | Z1 | KF999401 | KF999488 | This study |
| *E. v. velox* | 19 | Qitai , Xinjiang, China | 44.29°N, 90.11°E | WGXG08121 | Q3 | KF999402 | KF999489 | This study |
| *E. v. velox* | 19 | Qitai , Xinjiang, China | 44.29°N, 90.11°E | WGXG08122 | Q4 | KF999403 | KF999490 | This study |

**(Continued Appendix A)**

| Taxon | Locality No. | Geographical origin | Latitude, longitude | Sample No. | Haplotype type | Accession number | | Reference |
|---|---|---|---|---|---|---|---|---|
| | | | | | | cyt *b* | 12S rRNA | |
| *E. v. velox* | 20 | Tacheng, Xinjiang, China | 46.64°N, 82.95°E | JF1293 | TA1 | KF999395 | KF999482 | This study |
| *E. v. velox* | 21 | Fuyun, Xinjiang, China | 44.42°N, 88.81°E | JF1383 | Z1 | KF999393 | KF999480 | This study |
| *E. v. velox* | 22 | Gaoquan, Xinjiang, China | 44.48°N, 84.17°E | JF1230 | Z1 | KF999394 | KF999481 | This study |
| *E. v. roborowskii* | 23 | Turpan, Xinjiang, China | 42.86°N, 89.19°E | WGXG08202 | T1 | KF999332 | KF999419 | This study |
| *E. v. roborowskii* | 23 | Turpan, Xinjiang, China | 42.86°N, 89.19°E | WGXG08203 | T2 | KF999333 | KF999420 | This study |
| *E. v. roborowskii* | 23 | Turpan, Xinjiang, China | 42.86°N, 89.19°E | WGXG08204 | T1 | KF999334 | KF999421 | This study |
| *E. v. roborowskii* | 23 | Turpan, Xinjiang, China | 42.86°N, 89.19°E | WGXG08205 | T3 | KF999335 | KF999422 | This study |
| *E. v. roborowskii* | 23 | Turpan, Xinjiang, China | 42.86°N, 89.19°E | WGXG08206 | T1 | KF999336 | KF999423 | This study |
| *E. v. roborowskii* | 23 | Turpan, Xinjiang, China | 42.86°N, 89.19°E | WGXG08207 | T4 | KF999337 | KF999424 | This study |
| *E. v. roborowskii* | 23 | Turpan, Xinjiang, China | 42.86°N, 89.19°E | LJE0707006 | T5 | KF999372 | KF999459 | This study |
| *E. v. roborowskii* | 23 | Turpan, Xinjiang, China | 42.86°N, 89.19°E | LJE0707005 | T6 | KF999373 | KF999460 | This study |
| *E. v. roborowskii* | 23 | Turpan, Xinjiang, China | 42.86°N, 89.19°E | LJE0707004 | T7 | KF999374 | KF999461 | This study |
| *E. v. roborowskii* | 23 | Turpan, Xinjiang, China | 42.86°N, 89.19°E | LJE0707003 | T8 | KF999375 | KF999462 | This study |
| *E. v. roborowskii* | 23 | Turpan, Xinjiang, China | 42.86°N, 89.19°E | LJE0707002 | T9 | KF999376 | KF999463 | This study |
| *E. v. roborowskii* | 23 | Turpan, Xinjiang, China | 42.86°N, 89.19°E | LJE0707001 | T10 | KF999377 | KF999464 | This study |
| *E. v. roborowskii* | 24 | Jiaohe Ruins, Xinjiang, China | 42.95°N, 89.07°E | WGXG08208 | T11 | KF999338 | KF999425 | This study |
| *E. v. roborowskii* | 25 | Toksun, Xinjiang, China | 42.99°N, 88.75°E | WGXG08258 | T12 | KF999339 | KF999426 | This study |
| *E. v. roborowskii* | 25 | Toksun, Xinjiang, China | 42.99°N, 88.75°E | WGXG08259 | T12 | KF999379 | KF999465 | This study |
| *E. v. roborowskii* | 25 | Toksun, Xinjiang, China | 42.99°N, 88.75°E | WGXG08260 | T12 | KF999379 | KF999466 | This study |
| *E. v. roborowskii* | 25 | Toksun, Xinjiang, China | 42.99°N, 88.75°E | WGXG08261 | T12 | KF999380 | KF999467 | This study |
| *E. v. roborowskii* | 25 | Toksun, Xinjiang, China | 42.99°N, 88.75°E | WGXG08262 | T13 | KF999381 | KF999468 | This study |
| *E. v. roborowskii* | 25 | Toksun, Xinjiang, China | 42.99°N, 88.75°E | WGXG08263 | T12 | KF999382 | KF999469 | This study |
| *E. v. roborowskii* | 25 | Toksun, Xinjiang, China | 42.99°N, 88.75°E | WGXG08264 | T12 | KF999383 | KF999470 | This study |
| *E. v. caucasia* | 26 | Baskunchak, Astrakhan, Russia | 46.98°N, 48.21°E | Guo1266 | R1 | KF999340 | KF999427 | This study |
| *E. v. caucasia* | 27 | E Volga River, Astrakhan, Russia | 47.91°N, 46.57°E | Guo1268 | R1 | KF999341 | KF999428 | This study |
| *E. v. caucasia* | 27 | E Volga River, Astrakhan, Russia | 47.91°N, 46.57°E | Guo1269 | R1 | KF999342 | KF999429 | This study |
| *E. v. caucasia* | 27 | E Volga River, Astrakhan, Russia | 47.91°N, 46.57°E | Guo1270 | R2 | KF999343 | KF999430 | This study |
| *E. v. caucasia* | 27 | E Volga River, Astrakhan, Russia | 47.91°N, 46.57°E | Guo1271 | R1 | KF999344 | KF999431 | This study |
| *E. v. caucasia* | 27 | E Volga River, Astrakhan, Russia | 47.91°N, 46.57°E | Guo1272 | R1 | KF999345 | KF999432 | This study |
| *E. v. caucasia* | 27 | E Volga River, Astrakhan, Russia | 47.91°N, 46.57°E | Guo1273 | R1 | KF999346 | KF999433 | This study |
| *E. v. caucasia* | 27 | E Volga River, Astrakhan, Russia | 47.91°N, 46.57°E | Guo1274 | R3 | KF999347 | KF999434 | This study |
| *E. v. caucasia* | 28 | W Volga River, Astrakhan, Russia | 46.59°N, 47.88°E | Guo1285 | R3 | KF999350 | KF999437 | This study |
| *E. v. caucasia* | 28 | W Volga River, Astrakhan, Russia | 46.59°N, 47.88°E | Guo1286 | R6 | KF999349 | KF999436 | This study |
| *E. v. caucasia* | 28 | W Volga River, Astrakhan, Russia | 46.59°N, 47.88°E | Guo1287 | R7 | KF999348 | KF999435 | This study |
| *E. v. caucasia* | 28 | W Volga River, Astrakhan, Russia | 46.59°N, 47.88°E | Guo1289 | R1 | KF999357 | KF999444 | This study |
| *E. v. caucasia* | 28 | W Volga River, Astrakhan, Russia | 46.59°N, 47.88°E | Guo1290 | R1 | KF999356 | KF999443 | This study |
| *E. v. caucasia* | 29 | Kalmykia, Russia | 46.67°N, 46.29°E | Guo1276 | R4 | KF999355 | KF999442 | This study |

**(Continued Appendix A)**

| Taxon | Locality No. | Geographical origin | Latitude, longitude | Sample No. | Haplotype type | Accession number | | Reference |
|---|---|---|---|---|---|---|---|---|
| | | | | | | cyt *b* | 12S rRNA | |
| *E. v. caucasia* | 29 | Kalmykia, Russia | 46.67°N, 46.29°E | Guo1277 | R1 | KF999354 | KF999441 | This study |
| *E. v. caucasia* | 29 | Kalmykia, Russia | 46.67°N, 46.29°E | Guo1278 | R5 | KF999353 | KF999440 | This study |
| *E. v. caucasia* | 29 | Kalmykia, Russia | 46.67°N, 46.29°E | Guo1279 | R1 | KF999352 | KF999439 | This study |
| *E. v. caucasia* | 29 | Kalmykia, Russia | 46.67°N, 46.29°E | Guo1284 | R1 | KF999351 | KF999438 | This study |
| *E. v. caucasia* | 30 | Dagestan, Russia | 43.61°N, 46.34°E | Guo1711 | R8 | KF999391 | KF999478 | This study |
| *E. v. caucasia* | 30 | Dagestan, Russia | 43.61°N, 46.34°E | Guo1712 | R1 | KF999392 | KF999479 | This study |
| *Eremias* sp. 1 | 1 | North Tehran, Iran | – | 02-74 | E1 | JQ690169 | JQ690100 | * |
| *Eremias* sp. 2 | 3 | SE Golestan National park, Iran | – | Smp221 | E3 | JQ690175 | JQ690106 | * |
| *Eremias* sp. 2 | 6 | S Naishaboor, N Khorasan, Iran | – | EMP188 | E6 | JQ690187 | JQ690119 | * |
| *Eremias* sp. 3 | 2 | SE Tehran, Iran | – | ERP266 | E2 | JQ690172 | JQ690131 | * |
| *Eremias* sp. 3 | 4 | 10 km S Jajarm town, Iran | – | Smp268 | E4 | JQ690171 | JQ690102 | * |
| *Eremias* sp. 3 | 5 | 15 km SW Sabzevar, Iran | – | Smp15 | E5 | JQ690183 | JQ690115 | * |
| *E. velox* | 7 | S Aral Sea, Uzbekistan | – | Ev2 | UZ1 | JQ690216 | JQ690149 | * |
| *E. velox* | 7 | S Aral Sea, Uzbekistan | – | Ev3 | UZ2 | JQ690217 | JQ690150 | * |
| *E. velox* | 7 | S Aral Sea, Uzbekistan | – | Ev1 | UZ3 | JQ690218 | JQ690151 | * |
| *E. velox* | 7 | S Aral Sea, Uzbekistan | – | Ev4 | UZ4 | JQ690219 | JQ690152 | * |
| *E. velox* | 7 | S Aral Sea, Uzbekistan | – | Ev6 | UZ4 | JQ690221 | JQ690154 | * |
| *E. velox* | 7 | S Aral Sea, Uzbekistan | – | Ev7 | UZ5 | JQ690222 | JQ690155 | * |
| *E. velox* | 7 | S Aral Sea, Uzbekistan | – | Ev8 | UZ6 | JQ690223 | JQ690156 | * |
| *E. velox* | 7 | S Aral Sea, Uzbekistan | – | Ev5 | UZ6 | JQ690220 | JQ690153 | * |
| *E. velox* | 7 | S Aral Sea, Uzbekistan | – | Ev9 | UZ7 | JQ690224 | JQ690157 | * |
| *E. velox* | 7 | S Aral Sea, Uzbekistan | – | Ev11 | UZ8 | JQ690225 | JQ690158 | * |
| *E. velox* | 7 | S Aral Sea, Uzbekistan | – | Ev12 | UZ9 | JQ690226 | JQ690159 | * |
| *E. velox* | 7 | S Aral Sea, Uzbekistan | – | Ev13 | UZ10 | JQ690227 | JQ690160 | * |
| *E. v. caucasia* | 9 | Extreme W Kazakhstan | – | D4 | KZW1 | JQ690233 | JQ690166 | * |
| *E. v. velox* | 8 | W Aral Sea, Kazakhstan | – | M90 | KZ1 | JQ690212 | JQ690145 | * |
| *E. v. velox* | 10 | SW Balkhash Lake, Kazakhstan | – | K04-6 | KZ2 | JQ690215 | JQ690148 | * |
| *E. v. velox* | 10 | SW Balkhash Lake, Kazakhstan | – | D10 | KZ3 | JQ690228 | JQ690161 | * |
| *E. v. velox* | 10 | SW Balkhash Lake, Kazakhstan | – | D9 | KZ4 | JQ690229 | JQ690162 | * |
| *E. v. velox* | 10 | SW Balkhash Lake, Kazakhstan | – | RL26 | KZ5 | JQ690230 | JQ690163 | * |
| *E. v. velox* | 10 | SW Balkhash Lake, Kazakhstan | – | RL28 | KZ6 | JQ690231 | JQ690164 | * |
| *E. v. velox* | 10 | SW Balkhash Lake, Kazakhstan | – | RL65 | KZ7 | JQ690232 | JQ690165 | * |
| *E. v. velox* | 10 | SW Balkhash Lake, Kazakhstan | – | RL112 | KZ8 | JQ690234 | JQ690167 | * |
| *E. v. velox* | 11 | NE Aral Sea, Kazakhstan | – | 04-8 | KZ9 | JQ690213 | JQ690146 | * |
| *E. v. velox* | 11 | NE Aral Sea, Kazakhstan | – | K04-12 | KZ10 | JQ690214 | JQ690147 | * |
| *E. v. velox* | 12 | Turkistan, S Kazakhstan | – | T105 | KZ11 | JQ690206 | JQ690139 | * |
| *E. v. velox* | 13 | E Kazakhstan | – | M6 | KZ12 | JQ690204 | JQ690137 | * |
| *E. v. velox* | 13 | E Kazakhstan | – | U16 | KZ13 | JQ690205 | JQ690138 | * |
| *E. v. velox* | 13 | E Kazakhstan | – | Pp | KZ14 | JQ690207 | JQ690140 | * |
| *E. v. velox* | 13 | E Kazakhstan | – | A11 | KZ15 | JQ690208 | JQ690141 | * |
| *E. v. velox* | 13 | E Kazakhstan | – | A1 | KZ16 | JQ690209 | JQ690142 | * |
| *E. v. velox* | 13 | E Kazakhstan | – | U12 | KZ17 | JQ690210 | JQ690143 | * |
| *E. v. velox* | 13 | E Kazakhstan | – | M14 | KZ18 | JQ690211 | JQ690144 | * |
| *E. pesica* | – | Iran | – | ERP164 | – | FJ416285 | FJ445362 | * |
| *E. argus* | – | South Korea | – | LEGO-F415 | – | JQ086345 | JQ086345 | # |

* Rastegar-Pouyani *et al*. (2012); # Kim *et al*. (Unpublished data)